APPENDIX A

PATENT
Attorney Docket No.: 27373/39055B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wang *et al.*

Application No. 09/748,710

Filed: December 22, 2000

For: METHOD FOR GENERATION OF LONGER cDNA FRAGMENTS FROM SAGE TAGS FOR GENE IDENTIFICATION

Group Art Unit: 1637

Examiner: Joyce Tung

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

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WKM 2/19/04

Date: February 19, 2004

William K. Merkel

DECLARATION OF SAN MING WANG, JIAN-JUN CHEN, AND JANET ROWLEY UNDER 37 C.F.R. § 1.131

We, San Ming Wang, Jian-jun Chen, and Janet Rowley, state that,

1. We are the inventors of the subject matter claimed in the above-identified application, which claims priority to U.S. Provisional Application Nos.: 60/174,391 and 60/173,617, filed January 3, 2000, and December 29, 1999, respectively. We are making this declaration to provide evidence that the subject matter claimed in the above-identified application was completed in the United States at a date at least prior to July 19, 1999, which is the date of acceptance of van den Berg, et al., Nucleic Acids Research, 1999, Vol. 27(17), pages i-iii, cited by the Examiner in an Office Action mailed March 26, 2003.

2. To establish the date of completion of the invention, copies of pages from San Ming Wang's laboratory notebook are attached as Exhibit A. All dates appearing on these laboratory notebook pages have been redacted.

3. The laboratory notebook pages establish the following facts:

(a) Information from SAGE tags alone resulted in problems in identifying some genes;

(b) The solution to the problems associated with SAGE tags was to extend the SAGE tag sequence to generate a longer, more specific 3' cDNA probe;

(c) The longer probe could be generated using a SAGE tag as a primer in an extension and/or amplification reaction(s);

(d) One amplification reaction useful in solving the SAGE tag problem is PCR, using a SAGE tag primer and some form of universal primer, such as an oligo (dT) primer, optionally containing an anchor, with a cDNA as a substrate;

4. These laboratory notebook pages confirm that the subject matter of the pending claims in the application was completed at least prior to July 19, 1999.

5. Each of us hereby declares as follows: All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and, further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

San Ming Wang, Ph.D. Date 2.17.2004

Jian-jun Chen Date 2-18-2004

Janet Rowley Date 2-18-2004

Select candidate genes from different classes based on the Tag sequences from Lin Zhang, John Hopkins.

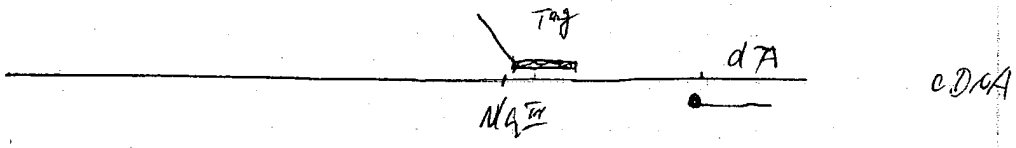
- Confirm the matched genes for Tag seq. by Blastn. parameter set: expect: 1000, Cutoff: 60. sequences aligned show many aligned genes in many cases to define the most likely ones, it needs to stress:

  a. 5' add CATG

  e.g. 5' CATG XXXXXXXXXX 3'
  Tag

  b. the matched sequences should be located in the last NlaIII site of the segment

  c. confirm the right gene by generally designing large templates based on the Tag sequence. This is not available in SAGE technique. I designed a system to stress this issue. If solved, it will largely improve the reality of SAGE gene identification in SAGE.



- based on Tag seq. design 5' seq (sense primer, plus 6 bases tail to maintain the stringency

anchored dT → It is know well Taq pol precisely distinguish e anchored primer reaction.

- use anchored primer as 3' antisense primer to select 3' subpopulation for PCR. IMPORTANT! NEW IDEA.
- PCR amplify cDNA
- the cDNA should be pre-digested by NlaIII. The possible outcome would be
- specific temp amplified because of the 5' sense primer / 3' anchored primer gives exponential amplification, others will only be ~~double~~ increased, 1, 2, 3, e— potential problem;
- amplification efficiency lower down to 3' extension consume large portion of substrants
- specificity ?

.CTED model system to confirm the possibility.

genes β-Actin S2/AS S2/ ~~Ati, S2/GdT~~ mixture of dT, A/G/C
HSC 70
HSP 75
HSP 78

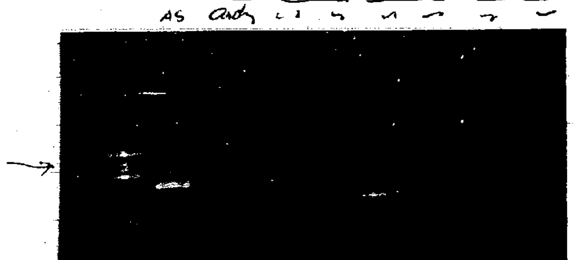
	1x	4x
H2O	31	124
MgCl2	5	20
buffer	5	20
dNTP	5	20
Taq	1	4
cDNA (dT, HL60 mRNA)	1	4

(Sense 2)

48	x8	x8	x8
1 (actin)	1 (70)	1 (75)	1 (78)

24	24	24	24	24	24	24	24
sense 0.5 (AS)	0.5 (mix anch. dT)	"	"	"	"	"	"

Actin 70 75 78
AS anch. " " " " " "



95 20"
50 20 } x5 → 60 20" } x25
72 20 — 95 20", 72 20"

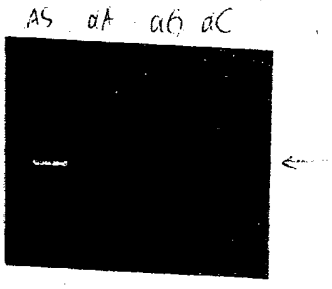
Conclusion. the mixture of anchored primers doesn't provide
[illegible]

The possible reason for failure to RT amplification may be that the mixture of anchor-dT distributed the efficient amplification. Try to use separated anchor-dT as 3' primer. use Actin as model

	2X
H_2O	6.2
$MgCl_2$	1.0
buffer	1.0
dNTP	1.0
Taq	0.2
cDNA	2
Sense (actin)	2

25 25 25 25

0.5 (AS) @.5 (dAT_n) 0.5 (dGT_n) 0.5 (dCT_n)



(β-actin mRNA end (HSACO7)

~~#~~AAGTG CACAC CTTA$_n$

ATTTTTTTTT ($dAGT_n$)

Conclusion: (1) dA anchor as 3' OK,

REDACTED

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Improve:

- increase 3' anchor primer amount
- decrease first 5 cycles in PCR to favor 3' primer bind to temp, then shift to 60°C for annealing
- increase cycle number to 40

	8x
MgCl2	40
buffer	40
dNTP	40
Taq	8
cDNA	8
Sense	8

9 × 15

9 — 9A — 9G — 9C

antisense 1 — 1 (50ng), 5 (250ng), 10 (500ng), 15 (750ng) — 15 10 15 — 1 5 10 15

H_2O 14 — 14 10 5 — 14 10 5 — 14 10 5 —

25 μl final

✓ Actin

✓ ef1-α

✓ ribosomal

✓ C37α

✓ S24

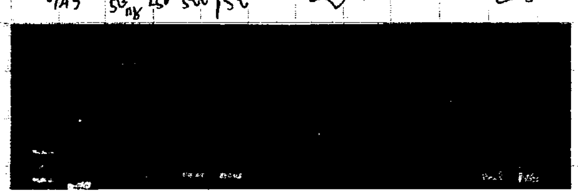
dATn
dTTn
dCTn

• X03767
M64013
U26726

AA5142
AJ193160
S28
AA67692
AA617970
AJ223473
D25786

94°C 20" / 42°C 20" / 72°C 20" } ×5 → 94°C 20" / 60°C 20" / 72°C 20" } ×30

S/AS	ATn				GTn	CTn
	50ng	250	500	750	~	~

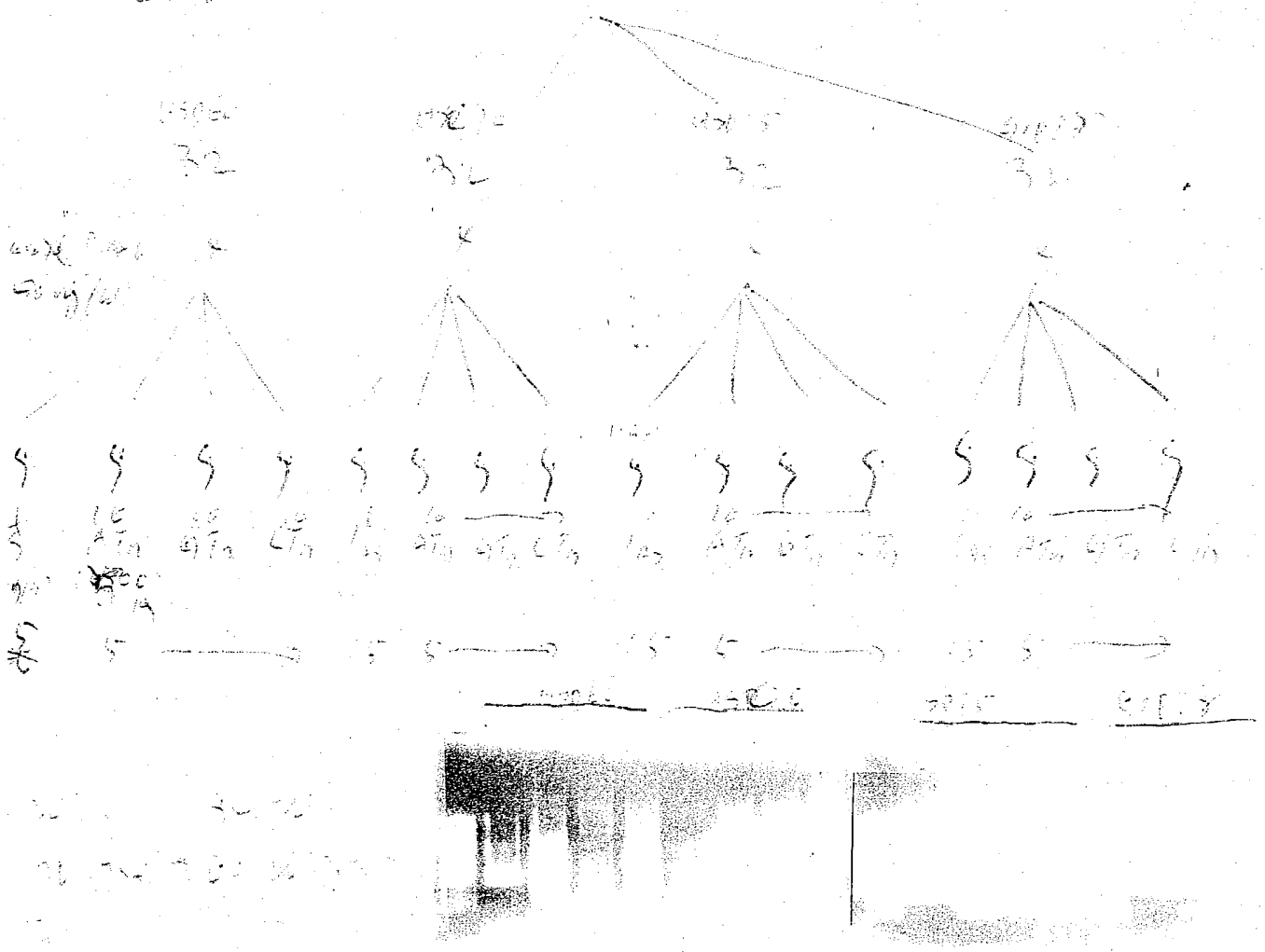


Conclusion:

① increase anchor primer amount did increase the amplification effi

② CTn still generated diffused

In principle, this strategy should work! Can be use

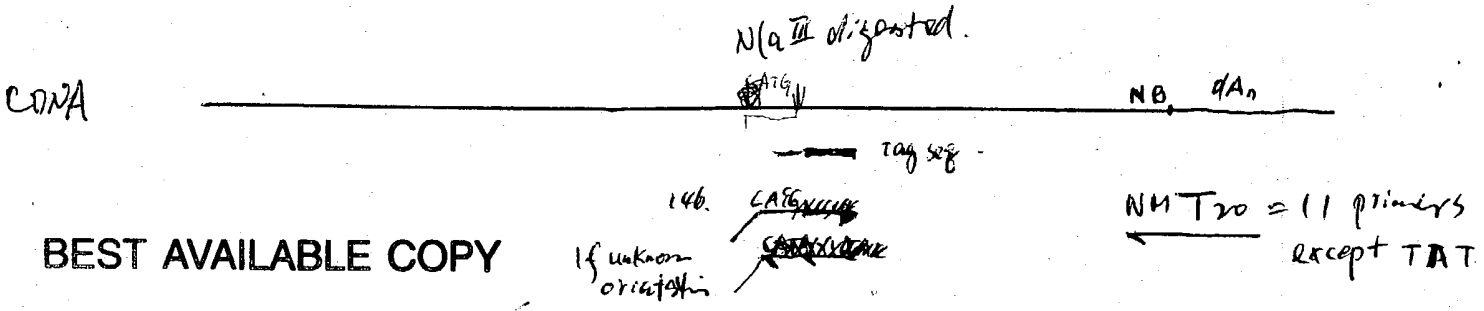


* also, the cDNA should be generated with anchor set not oligodT which creates background in PCR.

It seems that one base anchored dT primers may not generate enough specificity in particular temp, due to the complicated cDNA pool composition, or different members in the same family like HSP70s.

- TO increase the specificity, it may be useful to use 2 base anchored dT_n set as 3' antisense primer

The complete set of sense / antisense will be



- to identify each ~~temp~~ tag gene, the total reaction will be 11 PCR reaction with Tag seq as Sense (+ 6 base 5' te
- If negative, reversed Tag sequence will be used as the Sense primer in case the original Tag was generated in 3'-5 direction.
- in this way, the specific 3' fragment will be generated sequenced, and match to database.

t. try repeat exp. with anchor primer generated cDNA → { one base anch 5' S.